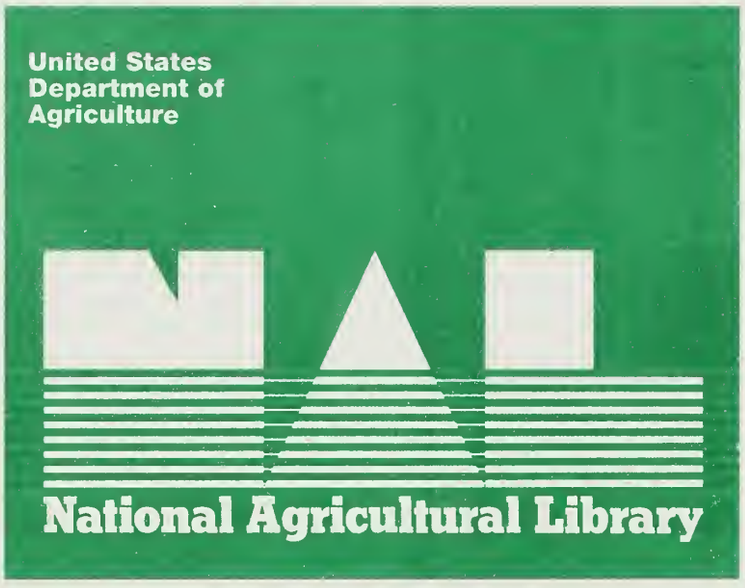
United States Department of Agriculture
Human Nutrition Research Center
at Grand Forks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AGRICULTURAL RESEARCH SERVICE

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

GRAND FORKS, NORTH DAKOTA

TABLE OF CONTENTS

United States Department of Agriculture
Agricultural Research Service
Northern Plains Area

Grand Forks Human Nutrition Research Center
2420 Second Avenue North
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Grand Forks, North Dakota 58202-7166

Mission Statement:

The Grand Forks Human Nutrition Research Center performs research designed to develop for humans recommended intakes of nutrients with emphasis on mineral requirements that will allow achievement of genetic potential and optimal function throughout the life cycle, and will provide information for decisions concerning the provision of a healthful food supply to the people of the United States. The five management units that accomplish this mission are entitled Office of the Center Director; Location Support; Clinical Nutrition; Nutrition, Biochemistry and Metabolism; and Nutrition, Physiology and Behavior.

Office of the Center Director

Mission:

The Office of the Center Director Management Unit provides leadership in the strategic planning and coordination of research programs, funding and staffing for the Grand Forks Human Nutrition Research Center.

Forrest H. Nielsen, Ph.D. Director (701) 795-8455	Provides leadership in the strategic planning and coordination of research programs, funding and staffing for the Center.
Beverly A. Shuler Secretary (701) 795-8456	Provides administrative assistance and secretarial support to to the Center Director.

Location Support

Mission:

The mission of the Location Support Management Unit is to provide a research environment that fosters creativity, the free exchange of ideas, and interdisciplinary research related to human nutrient requirements with emphasis on minerals. Thus, the Location Support Management Unit assures provision of a physical plant and equipment that allow the achievement of high standards in research quality and quantity. This Unit also provides biostatistical support for planning experiments and evaluation of data, and administrative support for servicing personnel needs, purchasing supplies and equipment, and maintaining financial records.

Phyllis Groven Supervisory Contract Specialist (701) 795-8444	Supervises all Location Support services. Administers contracts and agreements. Serves as Center Administrative Officer and Contracting Officer up to $100,000.00.
Deborah Meyer Accounting Technician (701) 795-8354	Maintains the location accounting system and assists in the preparation of the location budget. Reviews travel documentation and Time and Attendance records for completeness and accuracy.
Lauraine Troftgruben Purchasing Agent (701) 795-8412	Directs the daily operation of the purchasing section. Responsible for purchases up to $25,000 from open market sources and has unlimited authority to purchase from Federal Supply Schedules.
Darlyne Myrvik Purchasing Agent (701) 795-8445	Responsible for purchases up to $10,000 from open market sources and has unlimited authority to purchase from Federal Supply Schedules.
Gary Sagstuen Supply Clerk (701) 795-8469 - TDD	Functions as Head Cashier of the Imprest Fund. Types purchase orders and delivery orders. Receives incoming purchases and assists with the operation of the location stockroom.
Orris Johnson Electronic Engineer (701) 795-8422	Serves as Facility Manager responsible for installation of equipment and repair and maintenance needed by the location. Within delegation, provides inspection services for location construction projects.
Sherry Turner Clerk-Typist (701) 795-8370	Provides typing support for the Supervisory Contract Specialist. Processes and forwards personnel documents for the location to the Area Office and Headquarters. Prepares travel documents and Time and Attendance records for this Management Unit.

Clinical Nutrition

Mission:

The Clinical Nutrition Research Unit plans, implements and interprets research that is designed to produce new knowledge about human nutrient requirements with emphasis on mineral elements. The research will identify human needs necessary for achievement of genetic potentials and optimal function throughout the life cycle, and provide information for decisions concerning the provision of a healthful food supply to the U.S. population.

Human volunteers are studied under controlled conditions of a metabolic research ward or as free-living individuals to determine mineral element requirements and factors that influence requirements including interactions among nutrients, and between nutrients and non-nutrients that influence nutrient bioavailability and utilization.

Research Leader: Dr. Leslie M. Klevay

Analytical Biochemistry Laboratory

Mission:

To develop methods for the assessment of nutritional status with the emphasis on essential trace elements and vitamins that interact with these elements. To determine the effects of various dietary components on the requirements and metabolism of the essential trace elements.

David B. Milne, Ph.D. Research Chemist (701) 795-8424	Provides leadership to the laboratory. Develops methods for assessing trace element nutriture in humans and methods for quantitating vitamin and vitamin metabolites in human body fluids. Studies the effects of marginal intakes of copper and zinc and the interactions between trace elements and other nutrients in human subjects. Federal monitor for the Clinical Chemistry laboratory which provides analytical support for all human studies conducted at the Center.
Rodger Sims Chemist (701) 795-8425	Develops methods for and conducts trace element analyses of foods, tissues, balance samples and supplies used on the metabolic unit.

Recent Research Accomplishments:

Folic acid supplements of 400 μg every other day reduced urinary zinc excretion by 50% and increased fecal zinc losses in young men fed a diet marginal in zinc. This suggests that high dietary folic acid in relation to zinc impairs zinc absorption. Additional collaborative studies with pregnant women indicated that high blood folate and low plasma zinc were related to an increased risk of complications at delivery.

Made advances in sweat collection methods. With the advances showed that whole body sweat losses of zinc are a significant percentage of total zinc loss. Additionally, zinc loss in sweat declines with time during zinc depletion which suggests a homeostatic role for sweat in regulating zinc metabolism. These observations indicate that zinc lost in sweat is an important factor in determining human zinc requirements. Also showed that surface losses of copper of 1-6% of dietary intake may not be important for copper homeostasis.

Developed a method for the separation of platelets, mononucleated white cells, polynucleated white cells, and red blood cells on a discontinuous Percoll gradient. Apparent zinc content of the white cell fractions was dependent upon degree of separation from platelets. Subsequent studies with rats fed a severely zinc-deficient diet, and women fed a diet marginal in zinc, indicated no changes in the zinc content of blood cellular components. The findings suggest that zinc in blood cellular components is not a good indicator of zinc status. A procedure for measuring the manganese and copper content of blood cells, using graphite furnace atomic absorption spectrophotometry with Zeeman background correction, was developed. Erythrocytes accounted for about 66% of the total manganese in whole blood, whereas the "buffy coat" (platelets and leukocytes) accounted for about 30%. Because the "buffy coat" components turn over more rapidly than do erythrocytes, their manganese content may be a better indicator of manganese status.

Developed an isocratic HPLC procedure for the separation and quantitation of retinol (vitamin A), α-tocopherol (vitamin E), lycopene, α-carotene, and β-carotene, extracted from plasma. The small sample size, simplicity of extraction, short run time, accuracy, and reproducibility of the method make it ideal for use in either a clinical or research setting.

Found that ethanol metabolism was significantly impaired in postmenopausal women fed a diet containing 2.6 mg Zn/day for four months. The impairment seemed to be corrected within one month upon feeding adequate zinc. Plasma zinc and key zinc-containing enzymes were maintained throughout the low zinc intake period, either by strong homeostatic mechanisms or by shifting body pools of zinc. This suggests that functional aspects of zinc biochemistry, such as ethanol metabolism, may be more sensitive indicators of zinc nutriture and stores than circulating amounts of zinc.

A metabolic study with women fed a diet containing 0.67 mg copper/day and 1.5 g ascorbic acid/day indicated that cytochrome c oxidase in platelets and white cells, and the specific enzymatic activity of ceruloplasmin, may be more sensitive indicators of copper status than plasma copper or erythrocyte superoxide dismutase. Contrary to studies with laboratory animals, ascorbic acid supplements for six weeks did not markedly affect commonly measured indices of copper metabolism except for the specific enzymatic activity of ceruloplasmin.

Indices of iron status were evaluated in young women as they were being depleted of iron through low iron intake and phlebotomy. The relative sensitivities of different indices for detecting iron depletion were as follows: ferritin > % transferrin saturation > plasma iron > hemoglobin > zinc protoporphyrin and erythrocyte protoporphryn. Changes in heme synthesis evidently do not occur until iron stores are depleted and conversely, during iron repletion hematopoiesis must be satisfied before iron stores, as reflected by serum ferritin, increase. These findings indicate that one index of iron status is of limited value for detecting iron depletion. The use of two or more abnormal indices would better predict iron depletion.

Publications:

David B. Milne has collaborated on eight additional publications shown in the reference lists of the Absorption and Homeostasis of Trace Elements Laboratory; Applied Physiology Laboratory; Human Nutrient Requirements Laboratory; Trace Element Nutrition, Neuropsychological Function and Behavior Research Laboratory; Trace Elements and Cardiovascular Health Laboratory; and Ultratrace Elements Laboratory.

1990/1991

Milne DB, Sims RL, Ralston NVC. Manganese content of the cellular components of blood. *Clin Chem* 36: 450-452, 1990.

Milne DB, Gallagher SK, Nielsen FH. Response of various indices of iron status to acute iron depletion produced in menstruating women by low iron intake and phlebotomy. *Clin Chem* 36: 487-491, 1990.

Milne DB. The assessment of human copper nutritional status. *AACC Nutrition Division Newsletter* 8: 1-3, 1990.

Milne DB, Johnson PE, Klevay LM, Sandstead HH. Effect of copper intake on balance, absorption, and status indices of copper in men. *Nutr Res* 10: 975-986, 1990.

Sims RL, Mullen LM, Milne DB. Application of inductively coupled plasma emission spectroscopy to multielement analysis of foodstuffs used in metabolic studies. *J Food Comp Anal* (In press).

Milne DB, Lukaski HC, Johnson PE. Effect of folic acid supplements on zinc balance and metabolism in men fed diets adequate in zinc. *J Trace Elem Exp Med* (In press).

Milne DB, Nielsen FH, Lykken GI. Effects of dietary Cu and sulfur amino acids on Cu homeostasis and selected indices of Cu status in men. In: *Trace Element Metabolism in Man and Animals, TEMA-7* (In press).

Sandstead, HH, Dintzis FR, Bogyo TP, Milne DB, Jacob RA, Klevay LM. Dietary factors that can impair calcium and zinc nutriture of the elderly. In: *Nutrition and Aging.* DM Prinsley, HH Sandstead (eds). New York: Alan R Liss, Inc, pp 241-262, 1990.

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Gallagher SK, Johnson LK, Milne DB. Short-term and long-term variability of indices related to nutritional status. I. Ca, Cu, Fe, Mg, and Zn. *Clin Chem* 35: 369-373, 1989.

Milne DB. Effects of folic acid supplements on zinc-65 absorption and retention. *J Trace Elem Exp Med* 2: 297-304, 1989.

Ralston NVC, Schelkoph G, Milne DB. Alterations of physiological and biochemical blood cell indices by copper deficiency. *Proc ND Acad Sci* 43: 71, 1989.

Sims R, Ralston NVC, Milne DB. Distribution of copper and manganese in human blood and blood fractions. *Proc ND Acad Sci* 43: 84, 1989.

Metcoff J, Costiloe P, Crosby WM, Sandstead HH, Milne DB. Smoking in pregnancy: Relation of birth weight to maternal plasma carotene and cholesterol levels. *Obstet Gynecol* 74: 302-309, 1989.

Marchello MJ, Slanger WD, Milne DB, Fisher AG, Berg PT. Nutrient composition of raw and cooked bison bison. *J Food Comp Anal* 2: 177-185, 1989.

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Milne DB, Klevay LM, Hunt JR. Effects of ascorbic acid supplements and a diet marginal in copper on indices of copper status in women. *Nutr Res* 8: 865-873, 1988.

Justice PM, Kamath S, Langenberg PW, Sandstead HH, Milne DB, Smith GF. Micronutrients status of children with Down's syndrome: A comparative study of the effect of megadoses of vitamins with minerals or placebo. *Nutr Res* 8: 1251-1258, 1988.

Sims RL, Milne DB. Determination of manganese in whole blood and plasma using Zeeman graphite furnace AAS. *Proc ND Acad Sci* 42: 35, 1988.

Schelkoph GM, Milne DB. Microwave digestion of fecal samples for elemental analysis by inductively coupled plasma emission spectroscopy. *Proc ND Acad Sci* 42: 37, 1988.

Ralston NVC, Milne DB. Opposing effects of zinc and copper deficiencies in mean platelet volume. *Proc ND Acad Sci* 42: 37, 1988.

Schelkoph GM, Milne DB. Wet microwave digestion of diet and fecal samples for inductively coupled plasma analysis. *Anal Chem* 60: 2060-2062, 1988.

Milne DB, Klevay LM, Hunt JR. Comparison of indices of copper status in men and women fed diets marginal in copper. In: *Trace Element Metabolism in Man and Animals-6.* LS Hurley, CL Keen, B Lönnerdal, RB Rucker (eds). New York: Plenum Press, pp 451-452, 1988.

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Milne DB. Assessment of zinc and copper nutritional status in man: Some caveats. *Nutr and the MD* 13(5): 1-2, 1987.

Milne DB, Canfield WK, Gallagher SK, Mahalko JR, Klevay LM. Ethanol metabolism in postmenopausal women fed a diet marginal in zinc. *Am J Clin Nutr* 46: 688-693, 1987.

Sims RL, Mullen LM, Milne DB. Multielement analysis of foodstuffs using inductively coupled argon plasma. *Proc ND Acad Sci* 41: 78, 1987.

Ralston NVC, Theisen PW, Milne DB. Effects of hypertonic anticoagulants on the analytical determination of constituents in plasma. *Proc ND Acad Sci* 41: 81, 1987.

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Gautam D, Sinha RK, Milne DB. Interaction of Ponceau 4R with copper and effect of feeding Ponceau 4R on iron metabolism. *J Food Sci Tech* 23: 303-307, 1986.

Allison MJ, Cook HM, Milne DB, Gallagher S, Clayman RV. Oxalate degradation by gastrointestinal bacteria from humans. *J Nutr* 116: 455-460, 1986.

Milne DB, Botnen J. Retinol, α-tocopherol, lycopene, α- and β-carotene determined by isocratic liquid chromatography. *Clin Chem* 32: 874-876, 1986.

Ralston NVC, Theisen PW, Milne DB. Effect of platelet contamination on quantitation of leukocyte constituents. *Proc ND Acad Sci* 40: 79, 1986.

Gallagher SK, Johnson LK, Milne DB. Assessment of monthly, weekly, and daily variability of mineral indices of women fed constant or self-selected diets. *Proc ND Acad Sci* 40: 87, 1986.

Human Nutrient Requirements Laboratory

Mission:

To investigate human requirements for trace elements, especially iron and zinc; includes investigations of how nutrient requirements are influenced by individual diets, physiology, and lifestyles, and the effects of marginal deficiencies. To assess the nutrient content of diets by using computer-based nutrient data.

Janet R. Hunt, Ph.D., L.R.D. (formerly Janet R. Mahalko) Research Nutritionist (701) 795-8328	Provides leadership in human and animal studies on zinc and iron availability and requirements. Provides support in planning, implementing, and evaluating dietary aspects of human studies. Federal monitor for the Dietary Department that is under the supervision of the University of North Dakota employee, Bonita S. Hoverson, L.R.D., who manages food service operations, plans diets to meet the requirements of human research studies, manages the nutrient data base, and calculates nutrient content of diets for human studies.
Carol Ann Tekle-Wolde Chemist (701) 795-8375	Provides technical support in human and animal studies on zinc and iron availability and requirements.

Recent Research Accomplishments:

Demonstrated that zinc requirements increase with increased dietary protein intake, and that higher protein intakes result in greater bone zinc concentration in growing rats.

Determined that approximately one-fourth of the zinc in a representative U.S. diet is absorbed. Women who consume less total zinc absorb zinc more efficiently than men and retain a similar amount of zinc, adjusted for body weight.

Demonstrated that ascorbic acid supplements improve on-going iron absorption and retention in iron-depleted women consuming a diet with poorly available iron. This was the first demonstration that ascorbic acid can improve iron retention from a whole diet rather than just single meals.

Demonstrated that iron from soybean hulls is absorbed by humans as well as iron from bakery grade ferrous sulfate. Soybean hulls could be an economical and nutritious iron source for partial enrichment of bakery products.

Using the rat model, found that zinc availability from a variety of foods correlated with the amount of protein and several amino acids, but not the amount of phytic acid in the foods.

Developed a model demonstrating that zinc absorption by rats from a test meal is proportional to the natural log of the meal zinc content and the reciprocal of dietary zinc status; the greatest effects of zinc status on absorption are seen when low doses of zinc are used.

Publications:

Janet R. Hunt has collaborated on eleven additional publications shown in the reference lists of the Absorption and Homeostasis of Trace Elements Laboratory; Analytical Biochemistry Laboratory; and Ultratrace Elements Laboratory.

1990/1991

Hunt JR, Mullen LM, Lykken GI, Gallagher SK, Nielsen FH. Ascorbic acid: effect on on-going iron absorption and status in iron depleted young women. *Am J Clin Nutr* 51: 649-655, 1990.

Hunt JR, Larson BJ. Meal protein and zinc levels interact to influence zinc retention by the rat. *Nutr Res* 10: 697-705, 1990.

Hunt JR, Mullen LM, Lykken GI. Zinc retention by men and women consuming representative U.S. diets. *Seventh International Symposium on Trace Elements in Man and Animals* (In press).

Tekle-Wolde CA, Hunt JR. The effects of dietary protein intake on bone composition in the growing rat. *Proc ND Acad Sci* 44: 89, 1990.

Hunt JR, Mullen LM. Effect of energy intake on trace element balance. *Proc ND Acad Sci* 44: 66, 1990.

1989

Hunt JR, Johnson LK. The effect of dietary protein intake on zinc requirements and bone zinc in the growing rat. *Proc ND Acad Sci* 43: 54, 1989.

Hunt JR, Johnson PE, Swan PB. The dynamic nature of zinc availability from foods in vivo: Implications for in vitro methods. *Biol Trace Elem Res* 19: 119-127, 1989.

Hunt JR, Johnson PE, Swan PB. Effect of dietary zinc on Zn-65 absorption and turnover in rats. *Nutr Res* 9: 161-171, 1989.

Sandstead HH, Johnson L, Jacob RA, Hunt JR, Henriksen LK, Dintzis FR, Lykken GI, Johnson PE, Milne DB, Lukaski HC, Klevay LM. Bioavailability of zinc from human diets. In: *Bioavailability of Micronutrients and its Human Consequences*. EB High, WL Stone (eds). Nashville: Meharry Medical College, pp 23-32, 1989.

1988

Hunt JR. Egg white protein and zinc in a meal interact to affect zinc retention by rats. *Proc ND Acad Sci* 42: 65, 1988.

Hunt JR, Johnson PE, Swan PB. The effect of dietary Zn before and after 65-Zn administration on absorption and turnover of 65-Zn. In: *Trace Element Metabolism in Man and Animals-6.* LS Hurley, CL Keen, B Lönnerdal, RB Rucker (eds). New York: Plenum Press, pp 685-686, 1988.

Sandstead HH, Dintzis FR, Mahalko JR, Johnson LK, Bogyo TP. Effects of modest amounts of wheat bran and dietary protein on mineral metabolism of humans. In: *Trace Element Metabolism in Man and Animals-6.* LS Hurley, CL Keen, B Lönnerdal, RB Rucker (eds). New York: Plenum Press, pp 237-238, 1988.

1987

Lykken GI, Hunt JR, Nielsen EJ, Dintzis FR. Availability of soybean hull iron to humans in a mixed western meal. *J Food Sci* 52: 1545-1548, 1987.

Hunt JR, Johnson PE, Swan PB. Influence of usual zinc intake and zinc in a meal on 65-Zn retention and turnover in the rat. *J Nutr* 117: 1427-1433, 1987.

Hunt JR, Johnson P, Swan PB. The availability of zinc from foods fed to the rat. *Proc ND Acad Sci* 41: 52, 1987.

Hunt JR, Johnson PE, Swan PB. Dietary conditions influencing relative zinc availability from foods to the rat, and correlations with in vitro measurements. *J Nutr* 117: 1913-1923, 1987.

1986

Lykken GI, Mahalko J, Johnson PE, Milne D, Sandstead HH, Garcia WJ, Dintzis FR, Inglett GE. Effect of browned and unbrowned corn products intrinsically labeled with Zn-65 on absorption of Zn-65 in humans. *J Nutr* 116: 795-801, 1986.

Mahalko JR, Johnson PE, Swan PB. The influence of zinc dose, source, and status on zinc bioavailability in rats. *Proc ND Acad Sci* 40: 85, 1986.

Trace Elements and Cardiovascular Health Laboratory

Mission:

To define the effects of copper deprivation on the cardiovascular system. The effects of copper deprivation on organs, hormones and other metabolites that regulate cardiovascular physiology also will be studied. These studies will provide information useful in the definition of copper requirements. The effects of commonly consumed chemicals, both nutritive and non-nutritive, on copper requirements will be determined.

Leslie M. Klevay, M.D., S.D. in Hyg. Supervisory Research Medical Officer Research Leader (701) 795-8464	Leads research on cardiovascular growth, metabolism and function: Effects of copper, trace elements and modifying factors.
Sean M. Lynch Research Associate (701) 795-8405	Conducts research on the role of copper in blood coagulation/clot lysis related to hemorrhagic and hypercoagulability aspects of atherosclerosis.
Mary Rydell Secretary (701) 795-8464	Provides secretarial services and administrative assistance to the Research Leader and secretarial services for other scientists involved with research using human volunteers.

Recent Research Accomplishments:

Accomplishments include the following findings:

Copper deficiency lowers the activity of enzymes that affect cholesterol metabolism. Plasma lecithin:cholesterol acyltransferase and lipoprotein lipase activities were decreased in plasma of copper-deficient rats. As low activities of these enzymes lead to higher concentrations of plasma cholesterol, these findings, which have been confirmed by others, may explain partially the hypercholesterolemia of copper deficiency.

Copper deficiency impairs glucose metabolism. Copper-deficient rats had increased glycosylated hemoglobin, an indicator of elevated plasma glucose.

Clofibrate, a lipid-lowering drug, improves copper nutriture. Clofibrate will lessen the hypercholesterolemia of copper deficiency. The effect is mediated by an increase in liver copper. This observation led to the concept of cholesterotropic and cuprotropic chemicals. Some of these, e.g., aspirin, clofibrate and sodium phytate lower plasma cholesterol and enhance copper metabolism. Others, e.g., ascorbic acid, cholesterol plus cholic acid, and zinc, raise plasma cholesterol and inhibit copper metabolism.

Hypercholesterolemia and impaired glucose tolerance have been induced in men by feeding a low copper diet. Prolonged ingestion of a diet containing 0.8 mg of copper per day produced reversible increases in plasma cholesterol and the height of the glucose tolerance curve. Diets containing this amount of copper probably are consumed by 23% of the adult U.S. population. Hypercholesterolemia and glucose intolerance are common in the U.S. population.

Copper deficiency induces atrial thrombosis. For approximately 20 years, the adverse effects of certain diets on mice were attributed to the diets being high in fat. In reality, copper had been left out of the diets. Adequate copper prevented abnormal blood clotting and abnormal cardiograms and promoted far greater longevity. This

finding is similar to an earlier experiment in which a diet high in cholesterol had little effect on mice if dietary copper was adequate.

Abnormal electrocardiograms and hypercholesterolemia may be more sensitive indices of copper deficiency than anemia. Among non-anemic rats deficient in copper, abnormalities were found in the following (in order, beginning with greatest change): liver iron, heart dopamine, liver copper, plasma cholesterol, heart weight and heart norepinephrine.

Copper deficiency produces abnormal cardiac anatomy. Mitochondrial membranes deteriorated in hearts of rats deficient in copper; debris and vacuoles were seen. The collagen fibers that hold the cardiac muscle cells together were poorly developed. The activity of choline phosphotransferase was decreased in copper deficiency; this change may partially explain some of the anatomical changes noted.

The original observation (1973) linking copper metabolism and cholesterol metabolism has been confirmed in at least 15 independent laboratories. This paper is among the more frequently quoted from the American Journal of Clinical Nutrition and was the subject of a Citation Classic essay in Current Contents.

Extra dietary copper can abolish the hypercholesterolemia caused by feeding cholesterol plus cholic acid. Since 1924, cholesterol plus cholic acid have been fed to animals to induce atherosclerosis. This procedure induces copper deficiency in rats. Cholesterol fed to rabbits without cholic acid lowers liver copper and may induce copper deficiency. This method has been used since 1913 in the induction of atherosclerosis.

Adult rats deficient in copper are hypertensive; this new observation already has been confirmed. The decreased blood pressure of weanling rats made deficient in copper may be caused partly by decreased activity of angiotensin converting enzyme in plasma in addition to structural defects of heart and arteries.

A new way of explaining the clinical variability of specific human illnesses has been developed. Four classes of etiologic agents - toxicity, heredity, infection and deficiency are known. Illnesses that are caused by cooperating members of two classes have been identified; e.g., nutritional deficiency induced by a toxic agent. Three and four-way cooperations also exist; 15 cooperative mechanisms have been identified.

Kidneys of rats fed salt and deficient in copper fail with poor perfusion of blood and very large (greater than 90%) decrease in aldosterone and plasma renin. Kidney failure was produced with half the salt in half the time in comparison to classical experiments.

Rats fed a diet deficient in copper were given either beer or water to drink because of extensive data demonstrating that modest consumption of beer is associated with less death from heart disease than is abstinence from alcoholic beverages. Beer drinking rats live nearly six times as long with less heart damage and higher liver copper. Results were not from either the alcohol or the copper in beer; rather, animals absorbed and retained copper better.

Slight copper deficiency was induced in young pigs by feeding their mothers high doses of zinc during pregnancy; the normal conversion of cartilage into bone during development was retarded.

Diets in the U.S. seem to be low in copper in comparison to putative requirements. A unified theory is proposed that explains the high prevalence of ischemic heart disease in terms of dietary copper deficiency. Copper deficiency may induce this illness by weakening the connective tissue of arteries which are bathed in abnormal lipids and are under greater tension from high blood pressure. Arterial injury may be increased by decreased defense against oxidizing metabolites and by glycosylation of proteins.
More than 50 similarities between animals deficient in copper and people with ischemic heart disease have been identified. The most important of these are glucose intolerance, hypercholesterolemia, abnormal electrocardiograms, hyperuricemia, hypertension and the greater susceptibility of males.

Publications:

Leslie M. Klevay has collaborated on twelve additional publications shown in the reference lists of the Analytical Biochemistry Laboratory; Applied Physiology Laboratory; Cardiovascular Physiology Laboratory; Human Nutrient Requirements Laboratory; and Trace Element Nutrition, Neuropsychological Function and Behavior Research Laboratory.

1990/1991

Klevay LM. Motivation for cholesterol screening. *J Lab Clin Med* 115: 263, 1990.

Klevay LM, Moore RJ. Beer mitigates some effects of copper deficiency in rats. *Am J Clin Nutr* 51: 869-872, 1990.

Pond WG, Krook LP, Klevay LM. Bone pathology without cardiovascular lesions in pigs fed high zinc and low copper diet. *Nutr Res* 10: 871-885, 1990.

Klevay LM. Ischemic heart disease as copper deficiency. In: *Copper Bioavailability and Metabolism. (Adv Exp Med Biol, Vol 258)* Kies C (ed). New York: Plenum Publishing Corp, pp 197-208, 1990.

Klevay LM. Anatomy in sculpture and photography. *Sci Am* 262(2): 8, 1990.

Klevay LM. Ischemic heart disease: Toward a unified theory. In: *Role of Copper in Lipid Metabolism*. Lei KY, Carr TP (eds). Boca Raton, FL: CRC Press, pp 233-267, 1990.

Klevay LM. (Reviewer) Magnesium in Health and Disease by Y Itokawa and J Durlach. *J Am Dietet Assoc* 90: 1016, 1990.

Klevay LM, Halas ES. The effects of dietary copper deficiency and psychological stress on blood pressure in rats. *Physiol Behavior*, in press.

Klevay LM. Can copper deficiency cause ischemic heart disease? *Proc 7th International Symposium on Trace Element Metabolism in Man and Animals*, in press.

Klevay LM. Some environmental aspects of ischemic heart disease. *Environ Management and Health*, in press.

1989

Radhakrishnamurthy B, Ruiz H, Dalferes, ER, Jr, Klevay LM, Berenson GS. Composition of proteoglycans in the aortas of copper-deficient rats. *Proc Soc Exp Biol Med* 190: 98-104, 1989.

Moore RJ, Hall CB, Carlson EC, Lukaski HC, Klevay LM. Acute renal failure and fluid retention and kidney damage in copper deficient rats fed a high-NaCl diet. *J Lab Clin Med* 113: 516-528, 1989.

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Moore RJ, Klevay LM. Effect of copper deficiency on blood pressure and plasma and lung angiotensin-converting enzyme activity in rats. *Nutr Res* 8: 489-497, 1988.

Klevay LM. Dietary cholesterol lowers liver copper in rabbits. *Biol Trace Elem Res* 16: 51-57, 1988.

Klevay LM. Four ways of becoming ill. *Med Hypothesis* 27: 65-70, 1988.

Klevay LM. Cost effectiveness of antihyperlipemic therapy. *JAMA* 259: 1811, 1988.

Klevay LM. Insulin resistance in hypertension. *New Engl J Med* 318: 383, 1988.

Klevay LM. Ambivalence on rats. *Perspect Biol Med* 31: 588, 1988.

Klevay LM. Teeny-tiny energies. *Sky and Telecope* 76: 334, 1988.

Klevay LM. Beer increases the longevity of rats fed a diet deficient in copper. *Proc 6th International Symposium on Trace Element Metabolism in Man and Animals*. Hurley LS, Keen CL, Lönnerdal, Rucker RB (eds). New York: Plenum Press, pp 453-454, 1988.

1987

Klevay LM, Lykken GI. Reassurance regarding problems on Pennsylvania Avenue. *New Engl J Med* 316: 553-554, 1987.

Klevay LM. Scurvy as a deficiency disease. *Nutr Rev* 45: 126-127, 1987.

Klevay LM. Hypertension in rats due to copper deficiency. *Nutr Rep Int* 35: 999-1005, 1987.

Klevay LM. Cholesterol reduction: Safety and other concerns. *Ann Intern Med* 107: 421, 1987.

Klevay LM. Dietary requirements for trace elements in humans. *Proceedings of the Fourth International Workshop on Trace Element Analytical Chemistry in Medicine and Biology*. Bratter P, Schramel P (eds). 4: 43-60, 1987.

Klevay LM. Metals as nutritional factors. The IIIrd International Conference on Clinical Chemistry and Chemical Toxicology held in conjunction with the VIth International Symposium at the University of Occupational and Environmental Health. Kitakyusku, Japan, Proceedings: E Harwood Ltd., *J of the Univ of Occupational and Environmental Health 9*: Suppl, pp 59-72, 1987.

Klevay LM. Ischemic heart disease. A major obstacle to becoming old. *Clin Geriatric Med* 3: 361-372, 1987.

Klevay LM, Bistrian BR, Fleming CR, Neumann CG. Hair analysis in clinical and experimental medicine. *Am J Clin Nutr* 46: 233-236, 1987.

Klevay LM. Dietary copper: A powerful determinant of cholesterolemia. *Med Hypotheses* 24: 111-119, 1987.

Klevay LM. Dietary copper and human health. *Nutr & the MD* 13: 1-2 1987.

Klevay LM. This week's Citation Classic. (on *Am J Clin Nutr* 26: 1060, 1973). *Current Contents, Clinical Medicine* 28: 20, 1987.

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Cornatzer WE, Klevay LM. The effect of copper deficiency on heart microsomal phosphatidylcholine biosynthesis and concentration. *Int J Biochem* 18: 1083-1087, 1986.

Klevay LM, Canfield WK, Gallagher SK, Henriksen LK, Lukaski HC, Bolonchuk W, Johnson LK, Milne DB, Sandstead HH. Decreased glucose tolerance in two men during experimental copper depletion. *Nutr Rep Int* 33: 371-382, 1986.

Klevay LM. Aspirin hypocholesterolemia associated with increased microsomal copper in liver. *Nutr Res* 6: 1281-1292, 1986.

Nutrition, Biochemistry and Metabolism

Mission:

The Nutrition, Biochemistry and Metabolism Research Unit plans, implements and interprets biochemical and metabolic studies with humans and animals that provide insights into the reasons for the essentiality of various trace elements, and that show conditions under which regulation of metabolism is perturbed which provides information about situations in which the trace element content of the diet may be of concern. Research in this unit seeks to elucidate the metabolism of the mineral elements including the effects of mineral element deficiencies and excesses on other metabolites and the utilization of other nutrients; to define the effects of commonly ingested nutritive and non-nutritive materials on the metabolism of mineral elements; and to identify functional changes and/or adaptive mechanisms affecting responses to mineral elements. Research in this unit includes the role of copper in cell membrane function, the role of zinc in regulation of blood pressure and male reproductive function, homeostatic adjustments to changes in dietary zinc and in manganese, and the possible essentiality of certain elements such as boron and arsenic.

Research Leader: Dr. Phyllis E. Johnson

Absorption and Homeostasis of Trace Elements Laboratory

Mission:

To study factors affecting the absorption, retention and utilization of trace elements from food and diets and the homeostatic regulation of trace element metabolism, and to determine how these factors affect human nutrient requirements. This work includes development of methods for using stable isotopes in human studies and studies of the mechanisms by which trace element absorption and excretion occur. Studies in experimental animals (mainly rats) are used as preliminaries to studies in humans or to study factors which are inaccessible in humans.

Phyllis E. Johnson, Ph.D. Supervisory Research Chemist/Research Leader (701) 795-8416	Provides leadership in the areas of bioavailability, absorption and homeostasis of trace elements. Studies absorption of trace elements in humans by using radioisotopes or stable isotopes and mass spectrometry. Develops mass spectrometry methods and methods for isotopic labeling of foods. Studies factors affecting trace element absorption and retention by rats.
Diane Harless Secretary (701) 795-8355	Provides secretarial services and administrative assistance to the Research Leader and secretarial services for other scientists involved with research using animal models.
Richard Vanderpool, Ph.D. Research Chemist Postdoctoral Research Associate (701) 795-8486	Develops stable isotope methods for use of stable boron tracers.
John Finley, Ph.D. Research Chemist Postdoctoral Research Associate (701) 795-8486	Studies factors affecting excretion of zinc and manganese in bile and pancreatic fluid, and molecular speciation of zinc and manganese.
Eugene Korynta Biologist (701) 795-8415	Studies factors affecting manganese, zinc and copper absorption and excretion in animals.
Vacant Biological Aide (701) 795-84	Provides technical support, on a part-time basis, for the studies on the absorption, retention and utilization of trace elements.

Recent Research Accomplishments:

Found that Zn-65 incorporated intrinsically into beef is absorbed the same as Zn-65 added extrinsically to beef when the beef was fed as a hamburger with potatoes and a milkshake.

Found that manganese absorption in rats decreases as dietary manganese increases. The rate of manganese turnover differs depending on whether Mn-55 is administered orally or by injection.

Found that previous diet, which affected body zinc stores in rats, could affect zinc absorption and excretion; this effect occurred in addition to any effect of the current diet.

Developed an isotope dilution method for measurement of copper absorption and endogenous excretion by rats. Used this method to measure copper absorption and excretion in rats fed starch, glucose, fructose and sucrose. Rats fed starch absorbed significantly more copper than those fed the sugars.

Determined that injection of stable isotope Cu-65 into stems of wheat produces intrinsically labeled wheat that is physiologically the same as wheat with copper incorporated through normal growth processes. Produced wheat, peanuts and geese labeled intrinsically with Cu-65 for use in human studies.

Found that Cu-65 added intrinsically or extrinsically to wheat, goose meat, goose liver or peanut butter is absorbed equally from a typical American meal.

Found that women fed low manganese diets (~1 mg/day) had greater menstrual losses of manganese, iron and total hemoglobin than women fed 5.5 mg Mn/day. Dietary supplementation with calcium (1200 mg Ca/day) did not affect manganese absorption or turnover.

Found that intrinsic and extrinsic ^{54}Mn tracers are absorbed the same from plant foods by humans.

Found that dietary protein source does not significantly affect manganese bioavailability in rats.

Validated methodology for measuring zinc absorption and endogenous excretion in humans by using stable isotopes, and developed a method for measuring exchangeable zinc pools.

Found that 12-week old infants absorb zinc and copper more efficiently from breast milk than from infant formula, but net absorption of both zinc and copper is greater from formula because it contains higher amounts of these elements.

Publications:

Phyllis E. Johnson has collaborated on fifteen additional publications shown in the reference lists of the Analytical Biochemistry Laboratory; Cell Membrane Biochemistry Laboratory; Human Nutrient Requirements Laboratory; and Nutrition-Histopathology Laboratory.

1990/1991

Johnson PE, Nielsen FH. Copper, manganese, cobalt, and magnesium. In: *Meat and Health*. AM Pearson, TR Dutson (eds) (Adv Meat Res, Vol 6; Elsevier), pp 275-300, 1990.

Johnson PE. Factors affecting copper absorption in humans and animals. In: *Copper Bioavailability and Metabolism*. C Kies (ed). New York: Plenum, pp 71-79, 1990.

Johnson PE, Gallaher DD, Lykken GI, Hunt JR. Zinc availability from beef served with various carbohydrates or beverages. *Nutr Res* 10: 155-162, 1990.

Lee DY, Korynta E, Johnson PE. Effects of sex and age on manganese metabolism in rats fed Mn-supplemented or deficient diets. *Nutr Res* 10: 1005-1014, 1990.

Johnson PE, Korynta E. The effect of dietary protein source on manganese bioavailability to the rat. *Proc Soc Exp Biol Med* (In press, 1990).

Johnson PE. Effect of food processing and preparation procedures on mineral utilization. In: *Proceedings of Symposium on Nutritional and Toxicological Consequences of Food Processing.* M Friedman (ed). New York: Plenum (In press, 1990).

Vanderpool RA, Johnson PE. Thermal ionization mass spectrometry of boron. *Proc ND Acad Sci* 44: 92, 1990.

Johnson PE, Lykken GI, Korynta ED. Absorption and biological half-life of Mn-54 from intrinsically and extrinsically labeled foods in humans. *Proc ND Acad Sci* 43: 68, 1990.

Vanderpool RA, Johnson PE. Natural abundance and enriched boron isotope ratios in plant material. *Proc 38th Conf on Mass Spectrom Allied Topics*, pp 79-80, 1990.

Johnson PE, Vanderpool RA, Milne DB, Mahajan SK, Prasad AS, Mullen LK. Stable isotope studies of experimental zinc deficiency in adult men. *TEMA-7* (In press, 1990).

Johnson PE, Lykken GI. Manganese and calcium absorption and balance in young women fed diets with varying amounts of manganese and calcium. *J Trace Elem Exp Med* (In press, 1990).

Johnson PE, Lykken GI, Korynta ED. Absorption and biological half-life in humans of intrinsic and extrinsic ^{54}Mn tracers from foods of plant origin. *J Nutr* (In press, 1990).

1989

Johnson PE. Overview. What can in vitro methods tells us about mineral bioavailability? *Biol Trace Elem Res* 19: 3-10, 1989.

Lee DY, Johnson PE. ^{54}Mn absorption and excretion in rats fed soy protein and casein diets. *Proc Soc Exp Biol Med* 190: 211-216, 1989.

Johnson PE. Thermal ionization mass spectrometry of zinc in biological samples. *Proc ND Acad Sci* 43: 56, 1989.

Korynta ED, Johnson PE. The effects of animal protein on absorption and metabolism of manganese in the rat. *Proc ND Acad Sci* 43: 58, 1989.

Johnson PE. Methodology for stable isotope analysis in biological materials (A Review). *J Micronutr Anal* 6: 59-83, 1989.

Johnson PE, Canfield WK. Stable zinc and copper absorption in free-living infants fed breast milk or formula. *J Trace Elem Exp Med* 2: 285-295, 1989.

Johnson PE. The absorption and excretion of zinc in humans and animals. In: *Copper and Zinc in Inflammation*. R Milanino, KD Rainsford, GP Yelo (eds), pp 103-132, 1989.

1988

Johnson PE, Stuart MA, Bowman TD. Bioavailability of copper to rats from various foodstuffs and in the presence of different carbohydrates. *Proc Soc Exp Biol Med* 187: 44-50, 1988.

Johnson PE, Evans GW, Hunt JR. The effect of picolinic acid supplementation on zinc absorption by men fed a low tryptophan diet. *Nutr Res* 8: 119-127, 1988.

Gallaher DD, Johnson PE, Hunt JR, Lykken GI, Marchello M. Bioavailability in humans of zinc from beef: Intrinsic vs extrinsic labels. *Am J Clin Nutr* 48: 350-354, 1988.

Johnson PE, Lykken GI. ^{65}Cu absorption by men fed intrinsically and extrinsically labeled whole wheat bread. *J Agric Food Chem* 36: 537-540, 1988.

Johnson PE. Mean stool transit time. *Am J Clin Nutr* 48: 172, 1988.

Johnson PE, Hunt JR, Ralston NVC. The effect of past and current dietary Zn intake on Zn absorption and endogenous excretion in the rat. *J Nutr* 118: 1205-1209, 1988.

Johnson PE, Lee DY. Copper absorption and excretion measured by two methods in rats fed varying concentrations of dietary copper. *J Trace Elem Exp Med* 1: 129-142, 1988.

Johnson PE. The effect of various dietary carbohydrates on absorption and excretion of copper in the rat as measured by isotope dilution. *J Trace Elem Exp Med* 1: 143-156, 1988.

Lee DY, Johnson PE. Factors affecting absorption and excretion of ^{54}Mn in rats. *J Nutr* 118: 1509-1516, 1988.

Johnson PE, Stuart MA, Hunt JR, Mullen LM, Starks TL. ^{65}Cu absorption by women fed intrinsically and extrinsically labeled goose meat, goose liver, peanut butter and sunflower butter. *J Nutr* 118: 1522-1528, 1988.

1987

Johnson PE, Lukaski HC, Bowman TD. Effects of level and saturation of fat and iron level and type in the diet on iron absorption and utilization by the rat. *J Nutr* 117: 501-507, 1987.

Stuart MA, Johnson PE, Hamaker B, Kirleis A. Absorption of zinc and iron by rats fed meals containing sorghum food products. *J Cereal Sci* 6: 81-90, 1987.

Lee DY, Johnson PE. 54-Mn absorption and excretion in rats fed starch or sucrose. *Proc ND Acad Sci* 41: 53, 1987.

Hesse, LJ, Johnson PE. Copper absorption and status in rats fed varying levels of dietary copper. *Proc ND Acad Sci* 41: 92, 1987.

1986

Stuart MA, Johnson PE. Copper absorption and copper balance during consecutive periods for rats fed varying levels of dietary copper. *J Nutr* 116: 1028-1036, 1986.

Starks TL, Johnson PE. Evaluation of foliar application as techniques for intrinsically labeling wheat with Cu-65. *J Agr Food Chem* 34: 23-36, 1986.

Johnson PE, Shubert LE. Availability of iron from Spirulina, a blue-green alga. *Nutr Res* 6: 85-94, 1986.

Stuart MA, Johnson PE. Intrinsic labeling of confinement-reared goslings with Cu-65 for use in human absorption studies. *Nutr Res* 6: 203-213, 1986.

Bowman TD, Johnson PE. Radioisotope dilution method for the determination of true Cu absorption in the rat. *Proc ND Acad Sci* 40: 88, 1986.

Johnson PE. Implications of Folkers' speech. *Chem Engr News*, July 14, 1986.

Johnson PE, Shubert LE. Accumulation of mercury and other elements by Spirulina (Cyanophyceae). *Nutr Rept Intl* 34: 1063-1070, 1986.

Johnson PE. Trace minerals. In: *Food and Agricultural Research Opportunities to Improve Human Nutrition*. AR Doberenz, JA Milner, BS Schweigert (eds). Newark, DE: University of Delaware, pp A81-A83, 1986.

Cell Membrane Biochemistry Laboratory

Mission:

To determine the trace element requirements for maintaining normal biochemical functions of cell membranes. Emphasis is directed toward understanding the biochemical roles of copper and iron in maintaining cellular functions that are related to cell membranes. These functions include transmembrane signalling, cell proliferation and cell differentiation.

W. Thomas Johnson, Ph.D. Research Chemist (701) 795-8411	Provides leadership in conducting studies on the roles of trace metals in membrane biochemistry. Current research is directed towards understanding the mechanisms through which copper and iron influence signal-response coupling in platelets and cells in culture.
Steven N. Dufault Biologist (701) 795-8410	Provides technical support for studies on cell membranes in animals, humans and cultured cells.

Recent Research Accomplishments:

Demonstrated that copper deficiency increases the amount of a 170,000 dalton protein in erythrocyte membranes. This protein is associated with the cytoskeleton and indicates that copper may be essential for maintaining normal cytoskeletal structure and function in blood cells.

Demonstrated that interactions between cytoskeletal proteins in platelets following thrombin activation are affected by copper status. Myosin association with the cytoskeleton and actin polymerization following thrombin activation are enhanced in platelets from rats fed a copper-deficient diet when compared to rats fed adequate copper.

Found that dense granule secretion from thrombin-activated platelets is increased 2-fold by copper deficiency in rats. This hypersecretory response is apparently related to changes in the manner by which signals are processed by the protein kinase c-dependent signalling pathway. The specific defect may involve either depressed protein kinase c activity or impaired activation of this enzyme following platelet stimulation with thrombin.

Publications:

W. Thomas Johnson has collaborated on one additional publication shown in the reference list of the Cardiovascular Physiology Laboratory.

1990

Greeley S, Johnson WT, Schafer D, Johnson PE. Gestational alcoholism and fetal zinc accretion in Long-Evans rats. *J Am Coll Nutr* 9: 265-271, 1990.

Kramer TR, Johnson WT, Briske-Anderson M. Erythrocytes and latex particles enhance blastogenesis of concanavalin-A stimulated spleen lymphoid cells from copper-deficient rats. *Nutr Res* 10: 303-314, 1990.

1989

Johnson WT, Saari JT. Dietary supplementation with t-butylhydroquinone reduces cardiac hypertrophy and anemia associated with copper deficiency. *Nutr Res* 9: 1355-1362, 1989.

Johnson WT, Dufault SN. Altered cytoskeletal organization and secretory response of thrombin-activated platelets from copper-deficient rats. *J Nutr* 119: 1404-1410, 1989.

Dufault SN, Sakkinen PA, Johnson WT. Copper deficiency alters the response and cytoskeletal organization of thrombin-activated platelets. *Proc ND Acad Sci* 43: 44, 1989.

1988

Kramer TR, Johnson WT, Briske-Anderson M. Influence of iron and the sex of rats on hematological, biochemical and immunological changes during copper deficiency. *J Nutr* 118: 214-221, 1988.

1987

Johnson WT, Kramer TR. Effect of copper deficiency on erythrocyte membrane proteins in rats. *J Nutr* 117: 1085-1090, 1987.

Mayland HT, Kramer TR, Johnson WT. Trace elements in the nutrition and immunological responses of grazing livestock. In: *Proceedings, Grazing Livestock Nutrition Conference*, pp 101-113, 1987.

Davis MA, Johnson WT, Briske-Anderson M, Kramer TR. Lymphoid cell functions during copper deficiency. *Nutr Res* 7: 211-222, 1987.

Canfield WK, Johnson WT. The influence of the dietary ratio of polyunsaturated to saturated fatty acids on zinc metabolism. *Nutr Res* 7: 109-119, 1987.

1986

Johnson WT, Canfield WK. Intestinal absorption and excretion of zinc in streptozotocin diabetic rats as affected by dietary zinc and protein. *J Nutr* 115: 1217-1227, 1986.

Nutrition-Histopathology Laboratory

Mission:

To determine the essentiality, dietary requirement, interrelationships with other nutrients, and physiological function of boron, primarily through the use of histological techniques. To determine the effects of dietary boron, copper and zinc on bone morphology.

Curtiss D. Hunt, Ph.D. Research Biologist (701) 795-8423	Provides leadership in histological research. Studies the possible roles of dietary boron on bone and energy metabolism by using both animal models and human volunteers. Conducts animal studies on the roles of dietary zinc and copper in bone morphology and metabolism.
JoLayne Herbel Biologist (701) 795-8381	Conducts analytical and biochemical tests in studies concerned with the possible roles of boron in mineral and energy metabolism.

Recent Research Accomplishments:

Co-produced the first evidence that boron has an essential physiological role in the chick. In the chick, dietary boron affects several physiological indices including growth, tibial epiphysial growth plate calcification, and serum glucose. Previously, boron was considered essential only for plants.

Demonstrated that the effects of dietary boron on various morphological and biochemical indices are modified by the dietary concentrations of magnesium, calcium and cholecalciferol. In the cholecalciferol-deficient chick, supplemental dietary boron enhances growth at the expense of cartilage calcification when dietary magnesium is inadequate and slows growth to the benefit of calcification when dietary magnesium is adequate.

Identified a 5-fold range of boron intake that is beneficial to the cholecalciferol-deficient chick. A study with chicks indicated that optimum intake is about 1 μg B/g of dry diet. Further increases in supplemental dietary boron apparently overwhelm homeostatic controls.

Provided the first evidence that boron helps regulate energy metabolism in the rat. In the cholecalciferol-deficient rat, supplemental dietary boron markedly influenced key indicators of energy metabolism by decreasing plasma concentrations of creatine kinase, insulin and pyruvate, and by increasing plasma concentrations of thyroxine.

Provided the first evidence that boron protects the integrity of kidney function/structure from acute phase nephrotoxin-induced damage. Rats injected 24 hours previously with streptozotocin exhibited an abnormal elevation of total 24-hour urinary albumin, potassium and sodium; supplemental dietary boron alleviated the abnormal elevations. However, the protective influence of supplemental dietary boron was apparently overwhelmed with time; 48 hours after injection, compared to boron-deprived controls, the rats supplemented with dietary boron exhibited increased urinary excretion of those substances.

Developed a new low-temperature, wet-ashing method for use in boron analysis of biological tissues and fluids and foodstuffs. This method minimizes the loss of boron from the sample through volatilization. Volatilization is a significant problem in boron analysis methodology.

Determined that the daily intake of boron usually differs considerably between any two individuals by analyzing a variety of typical Western foods and personal care products. The concentration of boron in water varies considerably according to geographical source; at some locations the boron in drinking water and water-based beverages may account for most of the total dietary boron intake. Individual food preference greatly influences daily intake of boron; fruits, vegetables, tubers, and legumes have relatively much higher concentrations of boron than cereal grains or animal tissues and fluids. Also, boron is a significant contaminant of, or major ingredient of, many different personal care products.

Demonstrated that inadequate zinc nutriture during infancy, despite postlactational zinc repletion, induced imbalances in adult bone mineral metabolism when compared to zinc-adequate, pair-fed rat pups. At 150 days of age, zinc-deficient rat pups exhibited increased concentrations of bone phosphorus and magnesium and decreased concentrations of bone potassium.

Publications:

Curtiss D. Hunt has collaborated on two additional publications shown in the reference list of the Ultratrace Elements Laboratory.

1990/1991

Hunt CD, Shuler TR. Open-vessel, wet-ash, low-temperature digestion of biological materials for inductively coupled argon plasma spectroscopy (ICAP) analysis of boron and other elements. *J Micronutrient Anal* 6: 161-174, 1990.

Hunt CD, Shuler TR, Mullen LM. Concentration of boron and other elements in human foods and personal care products. *J Am Diet Assoc* (In press).

Hunt CD, Johnson PE. The effects of dietary zinc on human sperm morphology and seminal mineral loss. In: *Trace Element Metabolism in Man and Animals-7* (In press).

Hunt CD, Achen V, Johnson PE. Effects of short-term dietary zinc deficiency on sperm morphology and motility in humans. *Proc ND Acad Sci* 44: 65, 1990.

Herbel J, Hunt CD, Johnson PE. Effects of short-term dietary zinc deficiency on semen mineral concentrations in humans. *Proc ND Acad Sci* 44: 63, 1990.

Muessig KD, Hunt CD. Effects of boron, streptozotocin and their interaction on organ mineral concentrations in vitamin D_3-deficient rats. *Proc ND Acad Sci* 44: 74, 1990.

1989

Hunt CD. Dietary boron modified the effects of magnesium and molybdenum on mineral metabolism in the cholecalciferol-deficient chick. *Biol Trace Elem Res* 22: 201-220, 1989.

Hunt CD, Kalliokoski S, Herbel JL. Effects of boron, streptozotocin and their interaction on intermediate metabolism and bone turnover in rats. *Proc ND Acad Sci* 43: 53, 1989.

Achen V, Hunt CD. Use of an advanced image analysis system to determine the effects of dietary boron on bone morphology in the cholecalciferol-deficient chick. *Proc ND Acad Sci* 43: 37, 1989.

Herbel JL, Shuler TR, Ralston NVC, Hunt CD. Semi-closed, teflon tube, wet-ash digestion for the determination of boron in biological substances by inductively coupled argon plasma spectrophotometry. *Proc ND Acad Sci* 43: 52, 1989.

1988

Hunt CD, Halas ES, Eberhardt MJ. Long-term effects of lactational zinc deficiency on bone mineral composition in rats fed a commercially modified Luecke diet. *Biol Trace Elem Res* 16: 91-113, 1988.

Hunt CD, Nielsen FH. Dietary boron affects bone calcification in magnesium and cholecalciferol-deficient chicks. In: *Trace Element Metabolism in Man and Animals-6*. LC Hurley, CL Keen, B Lönnerdal, RB Rucker (eds). New York: Plenum Press, pp 275-276, 1988.

Hunt CD. Boron homeostasis in the cholecalciferol-deficient chick. *Proc ND Acad Sci* 42: 60, 1988.

1987

Hunt CD, Nielsen FH. Interactions among dietary boron, magnesium, and cholecalciferol in the chick. *Proc ND Acad Sci* 41: 50, 1987.

1986

Hunt CD, Nielsen FH. Dietary boron affects molybdenum and magnesium metabolism in the cholecalciferol-deficient chick. *Proc ND Acad Sci* 40: 83, 1986.

Peptide Hormone Metabolism and Cell Culture Laboratories

Mission:

To determine the biochemical function of zinc in man and animals. The emphasis for this research is placed on the role of zinc in metabolism and function of physiologically active peptides as acted upon by the zinc-dependent family of peptidases.

Philip G. Reeves, Ph.D. Supervisory Research Chemist (701) 795-8497	Provides leadership to the laboratory in animal and cell nutrition studies. Studies the effect of zinc status of animals and cells in culture on the function of peptide hormones and zinc-dependent peptidases that regulate blood pressure, food intake, and sexual development in the male.
Dennis Bobilya, Ph.D. Research Chemist Postdoctoral Research Associate (701) 795-8399	Directs independent studies on the mechanism of zinc-transport and metabolism in cultured animal cells
Mary Briske-Anderson Biologist (701) 795-8402	Provides technical support for the studies on zinc-dependent peptidases in cultured cells. Manages the cell culture laboratory.
Kerry Nelson Biologist (701) 795-8496	Provides technical support for the studies on zinc-dependent peptidases in animals. Manages the animal studies laboratory.
Lois Fredericks Biological Aide (701) 795-8402	Provides technical support, on a part time basis, for the studies on zinc-dependent peptidases in cultured cells.

Recent Research Accomplishments:

Established conditions for the growth and maintenance of cultured bovine pulmonary artery endothelial cells in a defined medium. Accomplished the first studies defining the mechanism of zinc transport in endothelial cells. Demonstrated that endothelial cells respond to high media zinc concentrations by producing metallothionein. Studied the effects of low zinc media on cell growth, morphology, and angiotensin-converting enzyme (ACE) activity. Isolated and grew bovine retinal capillary endothelial cells in culture.

Demonstrated that testosterone stimulates ACE activity in the testes of food-restricted rats but not in zinc-deficient rats of the same age. This suggests that the absence of zinc in the rat may specifically affect ACE protein synthesis.

ACE was isolated by affinity chromatography from rat testes and purified to a single protein. This protein will be used to produce antibodies for the study of zinc regulation of ACE synthesis in testes of rats.

Demonstrated that the hypothesis that metallothionein induction in kidneys by certain trace elements protects against kidney toxicity caused by cis-diamminedichloroplatinum, a cancer treatment drug, may not be tenable for all animals.

Publications:

Philip G. Reeves has collaborated on one additional publication shown in the reference list of the Cardiovascular Physiology Laboratory.

1990/1991

Reeves PG. Zinc deficiency and dipeptidyl carboxypeptidase activity: Comparative effects on epididymis and testes of rats. *Biol Tr Elem Res* 24: 1-11, 1990.

Reeves PG. Effects of zinc deficiency and testosterone treatment on the activities of dipeptidyl carboxypeptidase and other enzymes in the testis of rats. *Nutr Res* 10: 859-869, 1990.

Reeves PG, Noordewier B, Saari JT. Effect of copper deficiency and cis-diamminedichloroplatinum (II) treatment on the activities of renal microvillar enzymes in rats. *J Tr Elem Electrolytes Health Dis* 4: 11-19, 1990.

Reeves PG, Saari JT. Effect of cis-diamminedichloroplatinum (II) on metallothionein induction and trace element metabolism in rats fed different amounts of dietary zinc. *J Nutr Biochem* 1: 374-381, 1990.

Reeves PG, Nelson KL. Cis-diamminedichloroplatinum treatment and trace element metabolism in rats fed different amounts of dietary zinc and copper. *Proc Tr Elem Metab Man Ani* (In press, 1990).

Bobilya DJ, Briske-Anderson M, Reeves PG. Zinc uptake by endothelial cells. *Proc ND Acad Sci* 44: 52, 1990.

1989

Reeves PG. Effect of testosterone treatment on reproductive organ growth of Zn-deficient male rats. *Proc ND Acad Sci* 43: 72, 1989.

Reeves PG. AIN-76 diet: Should we change the formulation: Summary. *J Nutr* 119: 1081-1082, 1989.

1988

Reeves PG, O'Dell BL. Zinc deficiency in rats and angiotensin-converting enzyme activity: Comparative effects on lung and testis. *J Nutr* 118: 622-626, 1988.

Reeves PG. Effect of Zn deficiency on the activity of angiotensin converting enzyme in reproductive organs of testosterone-treated male rats. *Proc ND Acad Sci* 42: 66, 1988.

Reeves PG, O'Dell BL. Effect of zinc deficiency on blood pressure in rats fed normal and high levels of dietary calcium. *Nutr Res* 8: 1143-1150, 1988.

O'Dell BL, Reeves PG. Zinc status and food intake. In: *Zinc in Human Biology*. CF Mills (ed). London, United Kingdom: Springer-Verlag, pp 173-181, 1988.

1987

O'Dell BL, Browning JD, Reeves PG. Zinc deficiency increases osmotic fragility of rat erythrocytes. *J Nutr* 117: 1883-1889, 1987.

Browning JD, Reeves PG, O'Dell BL. Zinc deficiency in rats reduces the vasodilation responses to bradykinin and prostacyclin. *J Nutr* 117: 490-495, 1987.

1986

Reeves PG, O'Dell BL. Effect of dietary zinc deprivation on the activity of angiotensin converting enzyme in serum of rats and guinea pigs. *J Nutr* 116: 128-134, 1986.

Ultratrace Elements Laboratory

Mission:

To determine the essentiality, dietary requirement, utilization, interrelationships with other nutrients, and biochemical function of certain chemical elements, including arsenic, boron, nickel, silicon and vanadium, found in ultratrace amounts in food.

Eric O. Uthus, Ph.D. Research Chemist (701) 795-8382	Provides leadership to the laboratory. Studies the possible importance of arsenic and vanadium in nutrition by using animal models. Other ultratrace elements are also investigated if new knowledge suggests such studies might be fruitful. Chairman of the Animal Care Committee and Federal monitor of the Vivarium under the supervision of the University of North Dakota employee, Denice Schafer.
Forrest H. Nielsen, Ph.D. Supervisory Nutritionist (701) 795-8455	Studies the possible importance of boron and nickel in nutrition by using both animal models and human volunteers. Other ultratrace and trace elements are also investigated if new knowledge suggests such studies might be fruitful.
Carol D. Seaborn, Ph.D. Research Nutritionist Postdoctoral Research Associate (701) 795-8406	Investigates the biochemical, nutritional and physiological roles of silicon in animal models. Other ultratrace elements that might be involved in bone metabolism, including vanadium, are also investigated if there are indications such studies might be fruitful.
Terrence R. Shuler Chemist (701) 795-8364	Develops and modifies analytical methods for the determination of mineral elements in a variety of biological material. Assists with mineral element analyses emanating from experiments involving animal models.
Rhonda A. Poellot Biologist (701) 795-8406	Sets up, modifies and conducts analytical, biochemical and physiological tests in studies on the importance of ultratrace elements in nutrition.
Alyssa Martinez Biological Aide (701) 795-8406	Provides technical support, on a part time basis, for the studies on the importance of ultratrace elements in nutrition.

Recent Research Accomplishments:

Produced first evidence suggesting that nickel has an essential role in animals. Showed that nickel influences iron metabolism through physiological, pharmacological, and toxicological mechanisms. Also showed that vitamin B_{12} status and luxuriant amounts of an odd-chain fatty acid (margaric acid) in the diet affect the response of rats to nickel deprivation. These findings suggest that vitamin B_{12} may be necessary for the optimal expression of the biological role of nickel, which might involve the propionate pathway of branched-chain amino acid and odd-chain fatty acid metabolism. Thus, nickel might be of nutritional significance.

Co-produced the first evidence that boron is an essential nutrient for animals. Signs of boron deficiency were found to vary in nature and severity as diets varied in content of substances that affect macromineral metabolism, e.g., calcium, copper, and magnesium. Findings to date indicate that boron deprivation alters the function or composition of the skeleton, kidney, and brain. Prior to these findings, boron was considered essential only for plants. The findings established boron as an element to be considered in human nutrition.

Produced the first evidence showing that boron may be nutritionally important for humans. Findings were obtained indicating that the supplementation of a boron-low diet with an amount of boron commonly found in diets high in fruits and vegetables induces in postmenopausal women changes consistent with the prevention of calcium loss and bone demineralization. Boron nutriture is probably a contributing factor to some disorders characterized by abnormal mineral metabolism including osteoporosis.

Produced the first evidence suggesting that arsenic is an essential trace element. Demonstrated that dietary methionine and arginine markedly affect the response of chicks to arsenic deprivation. Also showed that arsenic deprivation in rats and chicks is affected by dietary manipulations affecting labile methyl metabolism. Produced the first evidence that arsenic deprivation decreases the concentration of liver polyamines in rats. The findings indicate that arsenic is important physiologically as a methylated compound or is involved in labile methyl metabolism. The findings should help in defining the essential role of arsenic and in determining whether arsenic is a practical human nutrition concern.

Produced the first evidence that dietary vanadium deprivation affects thyroid function of the rat. Vanadium deprivation elevates thyroid weight and depresses the activity of thyroid peroxidase. The findings suggest that vanadium might be essential for optimal thyroid function or thyroid hormone metabolism. These findings also should help determine whether vanadium is a practical nutritional concern for animals including humans.

Demonstrated that genetic factors, sex, and dietary sulfur amino acids markedly affect the nature and severity of the signs of copper deficiency in the rat and possibly humans. The findings indicate that the signs of copper deficiency in humans are not likely to be consistent, and have implications for the hypothesis that copper nutriture affects cardiovascular health.

Showed for the first time that experimentally significant effects other than on urinary magnesium can be obtained by the dietary restriction of magnesium in otherwise healthy adults. Magnesium deprivation apparently adversely affects the erythrocyte membrane and changes indices associated with bone metabolism. Magnesium may be of more nutritional concern than currently acknowledged.

Publications:

Forrest H. Nielsen has collaborated on eight additional publications shown in the reference lists of the Absorption and Homeostasis of Trace Elements Laboratory; Analytical Biochemistry Laboratory; Applied Physiology Laboratory; Human Nutrient Requirements Laboratory; and Nutrition-Histopathology Laboratory.

1990/1991

Uthus EO. Effects of dietary choline and methionine on arsenic deprivation in rats: Growth, organ weight/body weight ratios and hepatic polyamines. *Magnesium and Trace Elements* (In press).

Nielsen FH. Studies on the essentiality of some elements ascribed as toxic - arsenic, boron, lead, tin and vanadium. In: *Trace Elements in Man and Animals-7* (In press).

Nielsen FH, Milne DB. The effect of copper deprivation on variables associated with glucose metabolism in men. In: *Trace Elements in Man and Animals-7* (In press).

Nielsen FH. Chromium. In: *Modern Nutrition in Health and Disease, 8th ed.* ME Shils, JA Olson, M Shike (eds). Philadelphia: Lea & Febiger (In press).

Nielsen FH. Ultratrace elements. In: *Modern Nutrition in Health and Disease, 8th ed.* ME Shils, JA Olson, M Shike (eds). Philadelphia: Lea & Febiger (In press).

Nielsen FH. Ultratrace elements of possible importance for human health: An update. In: *Essential and Toxic Trace Elements in Human Health and Disease.* AS Prasad (ed). New York: Wiley (In press).

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Nutrition, Physiology and Behavior

Mission:

The Nutrition, Physiology and Behavior Management Unit plans, implements and interprets research that is designed to produce new knowledge about human nutrient requirements with an emphasis on trace elements. The research will identify human needs necessary for achievement of optimal physiological and psychological function and performance based on genetic potentials throughout the life cycle, and provide information for decisions concerning the provision of a healthful food supply to the population of the United States.

Animal models are used to provide information useful in planning studies with human volunteers, including safety, new analytical methods and demonstration of the usefulness of measuring previously ignored characteristics and responses. Information provided by the animal models will be used to elucidate the metabolism of the mineral elements, including the effects of mineral element deficiencies and excesses on other nutrients and to identify functional changes and/or adaptive mechanisms affecting responses to mineral nutrients. Research on animals provides the background for human studies by investigating hypotheses in ways that would be inappropriate for humans.

Human volunteers are studied under controlled conditions of a metabolic research ward or as free-living individuals to determine the effects of graded trace element intakes on physiological and psychological function and performance in response to static and dynamic stressors and challenges. The responses are evaluated and compared with standard and new biochemical indices of trace element status to identify impairments in function and performance. This information is necessary to determine previously unrecognized metabolic roles of essential and other trace elements.

Research Leader: Dr. Henry C. Lukaski

Applied Physiology Laboratory

Mission:

To determine the influence of alterations in trace element nutriture on static and dynamic physiological, metabolic and endocrine functions during acute and chronic exposure to stressors such as maximal and submaximal work and altered environmental conditions. To assess the effect of chronic high energy expenditure, with and without weight loss, on human trace element requirements. To develop and validate functional tests of nutritional assessment, and new approaches to assess human body composition. To determine the interactions among nutrition, body structure and function.

Henry C. Lukaski, Ph.D. Research Physiologist/Research Leader (701) 795-8429	Provides leadership to the laboratory. Studies relationships among metabolic, functional and biochemical responses to controlled stressors by animals and humans fed controlled diets containing graded amounts of trace elements. Develops noninvasive methods for assessing human body composition.
Clinton B. Hall, M.S. Biologist (701) 795-8495	Conducts physiological testing and body composition assessment of human volunteers and animals. Develops and modifies test protocols and equipment.
Scott M. Smith, Ph.D. Research Chemist Postdoctoral Research Associate (701) 795-8357	Conducts research on the role of trace elements and gender on energy metabolism and endocrine function.

Recent Research Accomplishments:

Developed and validated the tetrapolar bioelectrical impedance method for assessing human body composition. Models for predicting densitometrically-determined fat-free mass based upon conductance measurements of the body were developed and cross-validated in adults. The error of estimating fat-free mass was within the limits of the reference method of assessing body composition. The predictive accuracy was less than that found by standard anthropometric procedures. Other studies resulted in the development and validation of models using impedance measurements to estimate total body water and extracellular water in healthy adults. These relationships were subsequently used to assess fluid changes in surgical patients.

Provided first evidence that copper status affects human blood pressure. Significant increases in systolic and diastolic blood pressure responses during five minutes of isometric hand-grip exercise were exhibited by eight women consuming diets low in copper (0.65 mg/d) and supplemented with ascorbic acid (1.5 g/d). Resting blood pressures were not affected by low copper intake. These findings indicate a functional alteration in human blood pressure regulation during mild copper depletion.

Identified a unique biochemical adaptation associated with aerobic physical training. Significant increases in a copper- and zinc-containing enzyme, superoxide dismutase, responsible for destruction of oxygen free-radicals were observed in male and female collegiate swimmers after a competitive season. No changes were found in age-matched, non-training control subjects. The observed changes in the swimmers indicate a homeostatic response in copper metabolism to protect skeletal muscle from oxidative damage.

Developed and implemented a program for the prescription of aerobic exercise to maintain body composition and physical fitness of human volunteers residing on a metabolic unit. Use of this program facilitates control

of energy expenditure so that biochemical and physiological changes can be attributed with confidence to altered micronutrient intake.

Demonstrated that iron-deficiency without overt anemia in humans is associated with impairments in energy metabolism. A reduced thermogenic response during acute cold exposure and a blunted rate of oxygen utilization during progressive exercise to exhaustion were observed in 12 women who were made iron-deficient by a combination of reduced iron intake, phlebotomy and menstruation. It is noteworthy that the apparent inability to produce heat in the cold and reduced rate of oxygen uptake during work were associated with an increase in anaerobic metabolism. These data indicate that iron-dependent factors other than oxygen-carrying capacity can influence body metabolism.

Publications:

Henry C. Lukaski has collaborated on six additional publications shown in the reference lists of the Absorption and Homeostasis of Trace Elements Laboratory; Analytical Biochemistry Laboratory: Cardiovascular Physiology Laboratory; Human Nutrient Requirements Laboratory; and Trace Elements and Cardiovascular Health Laboratory.

1990/1991

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Hall CB, Lukaski HC, Marchello MJ. Estimation of rat body composition using tetrapolar bioelectrical impedance analysis. *Nutr Rep Int* 39: 627-623, 1989.

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Lukaski HC, Bolonchuk WW. Estimation of body fluid volumes using tetrapolar bioelectrical impedance measurements. *Aviat Space Environ Med* 59: 1163-1169, 1988.

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Lukaski HC. Potential uses of bioelectrical conductance methods. *Nutr Int* 3: 66, 1987.

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Lukaski HC. Use of the tetrapolar bioelectrical impedance method to assess human body composition. In: *Human Body Composition and Fat Distribution*. N Norgan (ed). Wageningen, Holland: EURO-NUT Report No. 8, pp 143-155, 1986.

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Bolonchuk WW, Lukaski HC. The relationship of skinfold thickness, bioelectrical impedance, and densitometric measurements to the components of somatotype. *Proc ND Acad Sci* 40: 56, 1986.

Siders WA, Lukaski HC, Bolonchuk WW. Body composition changes subsequent to physical training in college-age subjects: A meta-analysis. *Proc ND Acad Sci* 40: 125, 1986.

Cardiovascular Physiology Laboratory

Mission:

To determine the functional changes in heart, circulation and related systems caused by trace element deficiencies and, by use of physiological, pharmacological and biochemical techniques, to ascertain the mechanism of those changes.

Jack T. Saari, Ph.D. Research Physiologist (701) 795-8499	Provides leadership in studies determining the alteration of cardiovascular physiology in trace element-deficient animal models.
Gwen M. Schelkoph Chemist (701) 795-8498	Provides technical support for physiological studies of the cardiovascular system.

Recent Research Accomplishments:

Demonstrated that the administration of antioxidants to laboratory animals, either by feeding or injection, can inhibit the development of cardiac enlargement, cardiac edema and anemia caused by dietary copper deficiency.

Collaborated in a study which showed that ethane production, an indicator of lipid peroxidation, is enhanced when laboratory rats are fed a diet deficient in copper.

Demonstrated, in a collaborative study, that copper deficiency in rats enhances lung damage caused by breathing oxygen under high pressure. This further suggests that copper-deficient animals are vulnerable to oxidative stress.

Collaborated in research showing that microvascular function in rats is altered in copper deficiency. Specifically, copper deficiency enhanced the increase in microvascular permeability caused by release of histamine and inhibited adhesion of platelets to microvessel walls. This suggests that inflammation is enhanced and hemostatic (clotting) function is reduced in copper deficiency. Further studies of microvascular function showed that, though high blood cholesterol depressed copper status, the effects on inflammation and clotting were the opposite from those of copper deficiency.

Found that the aortas of copper-deficient rats are less capable of relaxing in response to agents that require an intact blood vessel endothelium for their action. This suggests a mechanism for the development of high blood pressure in copper deficiency.

Co-produced a series of studies illustrating that copper deficiency causes alteration of renal composition, enzyme activity, clearance function and body water retention. Further, renal damage by cis-platinum, an anti-tumor agent and nephrotoxin, is exaggerated in copper deficiency. Obtained evidence that cis-platinum acts by producing O_2-derived free radicals, which suggests that copper deficiency potentiates cis-platinum damage by reducing antioxidant defenses.

(The following accomplishments and publications reflect work by Jack T. Saari prior to his arrival in 1987 at the Grand Forks Human Nutrition Research Center.)

Demonstrated, by using electron microscopic techniques, that the calcium antagonist verapamil inhibits the binding of calcium to cellular membranes in the heart; this provides insight into the mechanism of action of this

important therapeutic drug. Using verapamil as a tool, showed that histamine enhances calcium binding to cardiac muscle and microvessel cellular membranes; these findings are consistent with the ability of this inflammatory agent to increase cardiac contractility and microvascular permeability.

Characterized, by pharmacological and biochemical techniques, the effect of histamine on coronary vessels of the rabbit heart. Illustrated the relative contribution of H_1 and H_2 receptors to contractile function of the vessels and the relationship of cyclic AMP to histamine-mediated vessel contraction. These findings are important in characterizing the potential of histamine to produce coronary vasospasm.

Publications:

Jack T. Saari has collaborated on three additional publications shown in the reference lists of the Cell Membrane Biochemistry Laboratory and the Peptide Hormone Metabolism and Cell Culture Laboratories.

1990

Saari JT, Schuschke DA, Ackermann DM, Miller FN. Effects of cholesterol feeding and copper deficiency on inflammation and thrombosis. *Proc ND Acad Sci* 44: 82, 1990.

Schelkoph GM, Saari JT. Detection of sulfite and sulfate in serum and urine using ion chromatography. *Proc ND Acad Sci* 44: 82, 1990.

Saari JT, Reeves PG, Noordewier B, Hall CB, Lukaski HC. Cardiovascular but not renal effects of copper deficiency are inhibited by dimethyl sulfoxide. *Nutr Res* 10: 467-477, 1990.

Schuschke DA, Saari JT, Ackermann DM, Miller FN. Progressive microcirculatory changes due to hypercholesterolemia in rats. *Am J Physiol* 258: H1464-H1469, 1990.

Saari JT, Dickerson FD, Habib MP. Ethane production in copper deficient rats. *Proc Soc Exp Biol Med* 195: 30-33, 1990.

1989

Schuschke DA, Saari JT. Histamine-mediated vasoconstriction and cAMP levels in coronary arteries of the isolated rabbit heart. *Pharmacology* 38: 23-33, 1989.

Saari JT. Chronic treatment with dimethyl sulfoxide protects against cardiovascular defects of copper deficiency. *Proc Soc Exp Biol Med* 190: 121-124, 1989.

Saari JT, Johnson WT. Inhibition of cardiovascular effects of copper deficiency with antioxidants. *Proc ND Acad Sci* 43: 80, 1989.

Schuschke DA, Saari JT, Ackermann DM, Miller FN. Microvascular responses in copper deficient rats. *Am J Physiol* 257: H1607-H1612, 1989.

1988

Saari JT, Klevay LM. Effect of dietary copper deficiency on vagal innervation of the heart. *Proc ND Acad Sci* 42: 63, 1988.

1987

Barman SA, Olson MD, Saari JT. Effect of histamine on cardiac sarcolemmal calcium binding as indicated by use of ionic lanthanum. *Cardiovascular Res* 21: 569-575, 1987.

Barman SA, Olson MD, Saari JT. Histamine-induced alteration of calcium binding to microvascular endothelium as indicated by use of ionic lanthanum. *Cardiovascular Res* 21: 576-581, 1987.

1986

Saari JT. Characterization of the coronary vascular response to histamine in rabbit hearts using cimetidine. *Pharmacology* 32: 80-89, 1986.

Trace Element Nutrition, Neuropsychological Function and Behavior Research Laboratory

Mission:

To apply psychological and neurophysiological principles and methods to the understanding of adult human behavior as influenced by nutrient intake and status, and to determine the dietary requirements for trace elements to achieve and maintain optimal cognitive performance and emotional adjustment in humans. To determine potential mediating factors in the nutrition-behavior relationship, including endogenous and exogenous stressors. To determine the effects of trace element nutrition on electrophysiology indexing cortical activity and autonomic activity to provide insight into the mechanisms for nutritional effects on psychological processes relevant to performance and adjustment. To develop methods of assessing behavior and neuropsychological responses in healthy adults which are sensitive to nutritional effects arising from marginal deficiencies and subclinical states.

James G. Penland, Ph.D. Research Psychologist (701) 795-8471	Provides leadership to the laboratory. Studies the relationships among trace element intake and status, behavior and psychophysiological responses in adult humans and animals. Determines the influence of boron, copper, iron, magnesium, manganese and zinc on brain function, cognitive performance, mood states and sleep behavior. Develops methods to assess psychological processes and behavior in the context of nutrition studies. Active in recruitment of human subjects for live-in studies and provision of counseling during their participation.
Gloria J. Krank, B.S. Psychologist (701) 795-8417	Coordinates and implements collection and analysis of behavioral and psychophysiological data in studies of trace element nutrition using humans and animals. Active in development and refinement of methods used to assess behavior and neurophysiological responses in humans and animals.

Recent Research Accomplishments:

Produced the first evidence that dietary boron may be important for brain function in animals. Electrocorticographic (ECoG) data were collected from 100-day old male and female rats fed either 0.12 or 2.71 μg/g boron for approximately 10 weeks. Low dietary boron decreased ECoG activity in both left and right hemispheres, particularly in the higher frequencies, and increased the proportion of lower frequency activity while decreasing the proportion of higher frequency activity.

Produced the first evidence that dietary boron may be important for brain function in healthy, older adults. In one study, electroencephalographic (EEG) data were collected from 13 healthy postmenopausal women fed either 0.23 or 3.23 mg/d boron as part of a 6-month metabolic unit study. Low dietary boron increased low-frequency EEG activity in parietal and left occipital regions of the head, and increased the proportion of lower to higher frequency activity, primarily in the frontal regions. In a second study, EEG data were collected from 10 healthy women (9 postmenopausal) and 5 healthy men older than 45 years fed either 0.23 or 3.23 mg/d boron as part of a 4-month free-living community study. Low dietary boron decreased EEG activity in the higher frequencies in the anterior regions of the head and in the lower frequencies in the posterior regions, and increased the

proportion of lower frequency activity while decreasing the proportion of higher frequency activity. In addition, low boron resulted in a greater left-minus-right hemisphere asymmetry in EEG activity.

Produced the first evidence that dietary boron may be important for sensory-motor function and cognitive performance. Data collected during the second study described above showed that the low boron intake resulted in impaired performance on tapping, pursuit, search, counting and encoding tasks.

Found that responses to a standardized self-report measure of mood states were related to dietary intakes and blood concentrations of aluminum, calcium, copper, iron, magnesium, manganese and zinc in healthy adult women participating in six independent, live-in studies of trace element nutrition. Dietary effects on mood states were evident in all six studies, with higher intakes of aluminum, copper and iron, and lower intakes of magnesium and zinc associated with more positive mood states. Correlations between mood states and concentrations of copper, iron, magnesium and zinc in the blood were numerous, but were often inconsistent when data from different studies were compared. Results were interpreted as providing only weak support for a trace element-mood relationship.

Determined sleep patterns in healthy, young adult women participating in several independent, live-in studies of trace element nutrition and related these patterns to dietary intakes of those elements. Compared to when they consumed > 2 mg/day copper, 11 women consuming < 1 mg/day copper reported earlier bedtimes, longer latency to sleep, longer total sleep time, and feeling less rested upon awakening. In contrast to when they were fed > 15 mg/day iron, 13 women fed < 5 mg/day iron reported earlier bedtimes, more nighttime awakenings, and longer total sleep time. Results were interpreted as providing moderate support for a trace element-sleep behavior relationship.

Found evidence of a possible relationship between dietary manganese and menstrual cycle symptomatology in a double-blind, cross-over study of dietary calcium and manganese effects in young women. Regardless of dietary calcium, low manganese intake (1 mg/d) resulted in increased reports of negative mood states during the menstrual and pre-menstrual phases of the cycle, and increased reports of pain during the pre-menstrual phase. Results suggest that low dietary manganese may produce increased behavioral responsiveness in menstruating women.

Produced the first evidence of a relationship between dietary copper and brain function in animals. ECoG data were collected from male rats fed 0.7, 1.4 or 2.7 μg/g copper for 100 days following weaning. Rats with the lowest copper intake showed decreased low-frequency amplitudes, increased middle- and higher-frequency amplitudes, and increased right-minus-left hemisphere asymmetries. Results suggest that copper deficiency may shift brain arousal and increase laterality of activity.

Found that when rat dams were fed <1 μg/g zinc during lactation, brain zinc/copper ratios of the mature, rehabilitated offspring were positively correlated with left-minus-right hemisphere asymmetries in brain electrical activity. Thus, zinc depletion during early development apparently may influence brain function well into adulthood, despite lengthy rehabilitation.

Developed a computer software package to automate the administration of standardized psychological tasks designed to assess a variety of cognitive processes (e.g., sensation, perception, attention, learning, memory, decision-making), spatial and sensory-motor skills in healthy adults. This system has been utilized in several studies of nutrition and cognition at both the Grand Forks and Western Human Nutrition Research Centers. Also developed and standardized several paper-and-pencil measures designed to assess mood states, stress, psychosocial behavior, daily activity levels, and sleep behavior of adults participating in metabolic unit and free-living studies of nutrition.

Found a relationship between several measures of iron status, cognitive performance and EEG parameters of healthy, older (>55 years) adults. Short-term memory for numerical sequences was poorer in individuals with

low iron status. Iron status was related to higher amplitudes in the lower EEG frequencies in the posterior regions of the brain, but to lower amplitudes in the higher frequencies recorded from anterior regions.

Publications:

1990/1991

Penland JG. Effects of dietary boron on the brain electrophysiology of healthy adults. *Am J Clin Nutr* (In press).

Tucker DM, Penland JG, Sandstead HH, Milne DB, Heck DG, Klevay LM. Nutritional status and brain function in aging. *Am J Clin Nutr* 52: 93-102, 1990.

Penland JG. Dietary boron affects brain function in mature Long-Evans rats. *Proc ND Acad Sci* 44: 78, 1990.

1989

Penland JG Sawler BG, Klevay LM. Brain electrophysiology in adult rats fed copper deficient diets. *J Trace Elem Exp Med* 2: 239-256, 1989.

Penland JG. Relationship between essential trace element nutrition and self-reported mood states. *Proc ND Acad Sci* 43: 68, 1989.

Health Care and Community Studies

Mission:

To work with principal investigators to design protocols appropriate for human studies and to ensure approved studies are implemented as planned. To ensure suitable participants are available for nutritional research on humans. To provide and/or monitor health care for research participants.

Donna Neese, R.N., B.S.N., M.S.
Nurse Consultant
(701) 795-8380

Federal monitor for the Metabolic Unit under the supervision of the University of North Dakota employee, Betty Vetter, R.N. Coordinates the development and processing of protocols for human studies. Coordinates the recruitment for live-in research participants. Develops and coordinates the Metabolic Unit test schedules according to the protocol and departmental needs and/or restrictions. Responsible for volunteer medical records. Is the Contracting Officer's Representative for the human subjects contract.

Emily Nielsen, R.N., B.S.N.
Nurse Specialist
(701) 795-8401

Recruits and screens free-living research participants. Develops schedules and coordinates community studies according to the research design, departmental needs and restrictions, and participant availability. Monitors participants' health status and study compliance. Principal contact person for participants in community studies. Assists in public relations including tours, presentations for the local community, and working with mass media.

Units Supervised by University of North Dakota Employees

The Federal staff of the Grand Forks Human Nutrition Research Center is supported by services from approximately 130 persons supplied through a Research Support Agreement with the University of North Dakota. The services include automatic data processing, janitorial, maintenance, routine laboratory, animal care, dishwashing, clinical laboratory, psychological testing, nursing, kitchen, dietary, recruiting, clerk-typist, and information processing. The supervisors of the units providing these services are shown below.

Supervisor	Service/Unit	Federal Monitor
Joan Flynn	Information Processing/Clerk-Typist	Beverly Shuler
	Store Clerk/Equipment Operator	Phyllis Groven
Sandra Gallagher	Clinical Laboratory	David Milne
	Routine Laboratory	Federal Support Staff
	Glassware	Forrest Nielsen
LuAnn Johnson	Data Processing	Forrest Nielsen
Bonita Hoverson	Dietary-Food Services	Janet Hunt
	Dietary-Nutrient Data Base	
Betty Vetter	Metabolic Unit	Donna Neese
Bonnie Thompson	Custodial	Phyllis Groven
Frances McSherry	Psychology	James Penland
Denice Schafer	Vivarium	Eric Uthus
Jerry Humble	Maintenance	Phyllis Groven
Joan Flynn	Recruiting	Donna Neese
	Overall Business Manager/Supervisor	Forrest Nielsen